LONGITUDINAL STUDY OF VAGINAL FLORA
REFERENCES & ABSTRACTS

NICHD Project

1Z01HD002535	Longitudinal Study of Vaginal Flora	FY99 – FY03
1ZIAHD002535	Studies of Vaginal Flora and Bacterial Vaginosis	FY04 – FY10

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Main Paper

1. Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu KF, Andrews WW. Vulvovaginal symptoms in women with bacterial vaginosis. *Obstet Gynecol* 2004;104(2):267-72.

Abstract: OBJECTIVE: A substantial, but highly variable, percentage of women with bacterial vaginosis are said to be asymptomatic. The purpose of this study was to estimate the prevalence of symptoms among women with bacterial vaginosis compared with women without bacterial vaginosis by direct, explicit, and detailed questioning of these women. METHODS: Women presenting for a routine health care visit at 12 health department clinics in Birmingham, Alabama, were recruited to participate in a longitudinal study of vaginal flora. At the first visit, they underwent a pelvic examination, lower genital tract microbiological evaluation, and an interview that included detailed questions regarding lower genital tract symptoms. The prevalence of symptoms among women with and without bacterial vaginosis (Gram stain score 7 or higher) was compared. RESULTS: Among 2,888 women without gonorrhea, Chlamydia, or trichomonas, 75% of women with and 82% of women without bacterial vaginosis never noted any vaginal odor in the past 6 months (P <.001). The corresponding values were 63% and 65% for never noting vaginal "wetness" (P =.02); 58% and 57% for vaginal discharge (P =.65); 91% and 86% for irritation (P =.004); 88% and 85% for itching (P =.64); and 96% and 94% for dysuria (P =.002), respectively. Cumulatively, 58% of women with bacterial vaginosis noted odor, discharge, and/or wetness in the past 6 months compared with 57% of women without bacterial vaginosis (P =.70). CONCLUSION: The 2 classic symptoms of bacterial vaginosis discharge and odor are each reported by a minority of women with bacterial vaginosis and are only slightly more prevalent than among women without bacterial vaginosis.

Additional Papers

2. Klebanoff MA, Andrews WW, Yu KF, Brotman RM, Nansel TR, Zhang J, Cliver SP, Schwebke JR. A pilot study of vaginal flora changes with randomization to cessation of douching. *Sex Transm Dis* 2006;33(10):610-3.

Abstract: OBJECTIVES: The objectives of this study were to determine whether women who douche regularly would enter a randomized trial of douching cessation or continuation, whether they would adhere to the assigned behavior, and whether there was a dramatic impact on vaginal flora. GOAL: The goal of this study was to determine the feasibility of a large, definitive trial of douching cessation. STUDY DESIGN: Women who douched at least weekly and who had either bacterial vaginosis or normal flora by Gram stain were assigned at random either to continue douching or to stop for 8 weeks. Vaginal Gram stains were obtained every 7 days from each woman. RESULTS: Forty-eight women were randomized. Those assigned to continue reported douching during 77% of study weeks; those assigned to stop denied douching in 94% of weeks. No dramatic differences in flora were observed between women in the continue versus stop groups. CONCLUSION: A large randomized trial of douching cessation is feasible.

3. Nansel TR, Riggs MA, Yu KF, Andrews WW, Schwebke JR, Klebanoff MA. The association of psychosocial stress and bacterial vaginosis in a longitudinal cohort. Am J Obstet Gynecol 2006;194(2):381-6.

Abstract: OBJECTIVE: The purpose of this study was to assess the association of psychosocial stress with bacterial vaginosis in a longitudinal sample of non-pregnant women. STUDY DESIGN: A 1-year prospective longitudinal design was used. Non-pregnant women (n = 3614) aged 15 to 44 years were recruited at routine health care visits. Assessments were conducted quarterly for 1 year and included a standardized pelvic examination, an assessment of clinical symptoms, and an extensive self-report interview. RESULTS: Psychosocial stress was associated with overall prevalence of (adjusted odds ratio, 1.10; 95% CI, 1.01-1.20) and an increased incidence of (adjusted odds ratio, 1.29; 95% CI, 1.12-1.48) bacterial vaginosis. The association between stress and bacterial vaginosis incidence was not changed appreciably by the control for behavioral and demographic characteristics and was magnified (odds ratio, 2.05; 95% CI, 1.15-3.66) in a case-crossover analysis. CONCLUSION: Increased psychosocial stress is associated with greater bacterial vaginosis prevalence and incidence independent of other risk factors.

4. Neggers YH, Nansel TR, Andrews WW, Schwebke JR, Yu KF, Goldenberg RL, Klebanoff MA. Dietary intake of selected nutrients affects bacterial vaginosis in women. *J Nutr* 2007;137(9):2128-33.

Abstract: Bacterial vaginosis (BV), a condition of altered vaginal flora, is associated with various adverse reproductive health outcomes. We evaluated the association between diet and the presence of BV in a subset of 1521 women (86% African-American) from a larger study of vaginal flora. Participants completed the Block Food Questionnaire and clinical assessments and self-report measures of sexual and hygiene behavior. A total of 42% of the women were classified as having BV (Nugent score ≥ 7). Severe BV (Nugent score ≥ 9 and vaginal pH ≥ 5) was present in 14.9% of the women. BV was associated [adjusted OR (AOR)] with increased dietary fat (1.5, 1.1-2.4) after adjusting for other energy nutrients and behavioral and demographic covariates. Severe BV was associated with total fat (2.3, 1.3-4.3), saturated fat (2.1, 1.2-3.9), and monounsaturated fat (2.2, 1.2-4.1). Energy intake was only marginally associated (P = 0.05) with BV (1.4, 1.0-1.8). There were significant inverse associations between severe BV and intakes of folate (0.4, 0.2-0.8), vitamin E (0.4, 0.2-0.8), and calcium (0.4, 0.3-0.7). We conclude that increased dietary fat intake is associated with increased risk of BV and severe BV, whereas increased intake of folate, vitamin A, and calcium may decrease the risk of severe BV.

5. Brotman RM, Klebanoff MA, Nansel T, Zhang J, Schwebke JR, Yu KF, Zenilman JM, Andrews WW. Why do women douche? A longitudinal study with two analytic approaches. *Ann Epidemiol* 2008;18(1):65-73.

Abstract: PURPOSE: Although vaginal douching is associated with several adverse outcomes, the reasons why women douche have not been studied prospectively. METHODS: Non-pregnant (N = 3620) women aged 15 to 44 years presenting for routine care at 12 clinics in Birmingham, Alabama, participated in a longitudinal study of vaginal flora (1999-2003). Participants were assessed quarterly for 1 year. The authors applied conditional logistic regression in a case-crossover analysis to determine the individual-level factors that vary between a woman's douching and non-douching intervals. Findings were compared to a population-level analysis utilizing generalized estimating equations. RESULTS: Thirty percent of participants douched in every interval; 28% douched in some but not all intervals. The case-crossover analysis indicated a woman was more likely to douche when reporting "fishy" vaginal odor (odds ratio [OR]:2.74; 95% confidence interval [CI]: 1.55, 1.84), vaginal irritation (OR: 1.52; 95% CI: 1.10, 2.11), summer month (OR: 1.37, 95% CI: 1.13, 1.67), or increase in number of sex partners (≥3, OR: 2.42, 95% CI: 1.11, 5.26). Bacterial vaginosis/trichomoniasis treatment (OR: 0.72, 95% CL: 0.59, 0.89) and absent menses (OR: 0.37, 95% CI: 0.28, 0.50) were negatively associated with douching. These ORs were farther from the null than comparable population-level estimates. CONCLUSIONS: Programs targeting these predictors may decrease the untoward sequelae associated with douching. Furthermore, a case-crossover analysis applied to prospective studies can provide insights into time-varying factors.

6. Brotman RM, Klebanoff MA, Nansel TR, Andrews WW, Schwebke JR, Zhang J, Yu KF, Zenilman JM, Scharfstein DO. A longitudinal study of vaginal douching and bacterial vaginosis - a marginal structural modeling analysis. *Am J Epidemiol* 2008;168(2):188-96.

Abstract: The etiology of bacterial vaginosis is unknown, and there are no long-term therapies for preventing this frequently recurring condition. Vaginal douching has been reported to be associated with bacterial vaginosis in observational studies. However, this association may be due to confounding by indication--that is, confounding by women douching in response to vaginal symptoms associated with bacterial vaginosis. The authors used marginal structural modeling to estimate the causal effect of douching on bacterial vaginosis risk while controlling for this confounding effect. In 1999-2002, non-pregnant women (n = 3,620) were recruited into a prospective study when they visited one of 12 public health clinics in Birmingham, Alabama, for routine care. Participants were assessed quarterly for 1 year. Bacterial vaginosis was based on a Nugent's Gram stain score of 7 or higher. Thirty-two percent of participants douched in every study interval, and 43.0% never douched. Of the 12,349 study visits, 40.2% were classified as involving bacterial vaginosis. The relative risk for regular douching as compared with no douching was 1.21 (95% confidence interval: 1.08, 1.38). These findings indicate that douching confers increased risk of disruption of vaginal flora. In the absence of a large randomized trial, these findings provide the best evidence to date for a risk of bacterial vaginosis associated with douching.

7. Klebanoff MA, Nansel TR, Brotman RM, Zhang J, Yu KF, Schwebke JR, Andrews WW. Personal hygienic behaviors and bacterial vaginosis. *Sex Transm Dis* 2010;37(2):94-9.

Abstract: BACKGROUND: Vaginal douching is consistently associated with bacterial vaginosis (BV), but whether it is a cause or result of BV remains unknown. The association between BV and other feminine hygienic behaviors is less studied; if BV symptoms caused behavior change then all hygiene behaviors might be more common among women with BV. Lack of association between non-douching hygiene behavior and BV would argue against reverse causation. METHODS: In the Longitudinal Study of Vaginal Flora 3620 women had 13,517 visits where BV (Nugent score) was assessed. Associations between hygienic behavior and BV were assessed by Poisson regression. RESULTS: After adjusting for demographic and sexual behavior factors, neither type of underwear (nylon vs. cotton prevalence ratio (PR) 1.05, 95% CI: 0.97-1.13), menstrual protection (tampons vs. pads; PR: 1.04, 95% CI: 0.95-1.12; pads and tampons vs. pads 1.00, 95% CI: 0.92-1.07), use of pads or panty liners when not menstruating (PR: 0.99, 95% CI: 0.95-1.05), nor weekly or greater use of hygiene spray (PR: 1.01, 95% CI: 0.94-1.09), powder (PR: 1.02, 95% CI: 0.96-1.07) or towelettes (PR: 1.03, 95% CI: 0.94-1.13) were strongly associated with BV. PR for daily versus less than daily bathing and showering were 1.06 (95% CI: 1.02-1.12) and 1.04 (95% CI: 1.00-1.09). Douching remained associated with BV (PR for weekly or greater vs. never 1.17, 95% CI: 1.09-1.26) and was not substantially impacted by adjustment for other hygienic behavior. CONCLUSIONS: Douching, but not other feminine hygiene behaviors, is significantly associated with BV, providing additional evidence that douching may be causally associated with BV and is not simply a response to BV symptoms.

8. Klebanoff MA, Andrews WW, Zhang J, Brotman RM, Nansel TR, Yu KF, Schwebke JR, Andrews WW. Race of male sex partners and occurrence of bacterial vaginosis. *Sex Transm Dis* 2010;37(3):184-90.

Abstract: BACKGROUND: Whether bacterial vaginosis (BV) is sexually transmitted is uncertain. Also it is unknown why BV is approximately twice as prevalent among black as among white women. An association of BV with a characteristic of the male sex partner, such as race, might support sexual transmission as well as account for the observed ethnic disparity in BV. METHODS: Three thousand six hundred twenty non-pregnant women 15 to 44 years of age were followed quarterly for 1 year. At each visit, extensive questionnaire data and vaginal swabs for Gram's staining were obtained. The outcome was transition from BV-negative to positive (Nugent's score ≥7) in an interval of 2 consecutive visits. RESULTS: BV occurred in 12.8% of 906 sexually active intervals to white women-24.8% of intervals when the woman reported a black partner and 10.7% when all partners were white. Among white women, there was a 2-fold increased risk for BV incidence with a black, compared with a white partner (risk ratio [RR] 2.3, 95% confidence interval 1.6-3.4; adjusted RR 2.2, 95% confidence interval 1.5-3.4), but differed according to condom use. In the presence of consistent condom use, the adjusted RR was 0.7 (0.3-2.4); it was 2.4 (1.0-6.2) in the presence of inconsistent use; and 2.7 (1.7-4.2) in the absence of condom use. Black women could not be studied, as there were insufficient numbers who reported only white male sex partners. CONCLUSION: The association of BV occurrence with partner's race, and its blunting by condom use, suggests that BV may have a core group component and may be sexually transmitted.

9. Brotman RM, Klebanoff MA, Nansel TR, Yu KF, Andrews WW, Zhang J, Schwebke JR. Bacterial vaginosis assessed by Gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *J Infect Dis* 2010;202(12):1907-15.

Abstract: BACKGROUND: We sought to assess the relationship between bacterial vaginosis (BV) assessed by Gram stain and incident trichomonal, gonococcal, and/or chlamydial genital infection. METHODS: This longitudinal study included 3620 non-pregnant women aged 15-44 years who presented for routine care at 12 clinics in Birmingham, Alabama. Participants were assessed quarterly for 1 year. Vaginal smears were categorized by the Nugent Gram stain score (0-3, normal; 4-6, intermediate state; 7-10, BV). Pooled logistic regression was used to estimate the hazard ratios for the comparison of trichomonal, gonococcal, and chlamydial infection incidence in participants by Nugent score at the prior visit. Participants were censored at their first visit with a positive test result for trichomonal, gonococcal, and/or chlamydial infection. RESULTS: Of the 10,606 eligible visits, 37.96% were classified by BV and 13.3% by positive detection of trichomonal, gonococcal, and/or chlamydial infection. An intermediate state or BV at the prior visit was associated with a 1.5-2-fold increased risk for incident trichomonal, gonococcal, and/or chlamydial infection (adjusted hazard ratio [AHR] for intermediate state, 1.41 [95% confidence interval {CI}, 1.12-1.76]; AHR for BV, 1.73 [95% CI, 1.42-2.11]; P= .058 for trend). Estimates were similar for trichomonal-only, gonococcal-only, and chlamydial-only infection outcomes. CONCLUSION: BV microbiota as gauged by Gram stain is associated with

a significantly elevated risk for acquisition of trichomonal, gonococcal, and/or chlamydial genital infection.

10. Thoma ME, Klebanoff MA, Rovner AJ, Nansel TR, Neggers Y, Andrews WW, Schwebke JR. Bacterial vaginosis is associated with variation in dietary indices. *J Nutr* 2011;141(9):1698-704.

Abstract: Bacterial vaginosis (BV) is a common condition of unknown etiology and has been linked to adverse reproductive and obstetric health outcomes. Prior dietary research on BV has focused on specific macro- and micronutrients, but not dietary indices. We assessed the relationship between BV and selected dietary indicators among a cohort of 1735 non-pregnant women ages 15-44 y from Birmingham, Alabama. Annual intake was assessed with the Block98 FFQ, and the glycemic index, glycemic load (GL), and Healthy Eating Index were calculated by the Block Dietary Data System. The Naturally Nutrient Rich (NNR) score was also calculated. Vaginal flora was evaluated using Nugent Gram-stain criteria. Crude OR and adjusted OR were determined by multinomial and logistic regression in cross-sectional and prospective analyses, respectively. Participants were predominantly African American (85.5%) aged 25.3 ± 6.8 y (mean ± SD). Per 10-unit increase, GL was positively (adjusted OR = 1.01, 95% CI = 1.00-1.03) and NNR was negatively (adjusted OR = 0.93, 95% CI = 0.88-0.99) associated with BV compared to normal vaginal flora. In prospective analyses, only GL was associated with BV progression (adjusted OR = 1.03, 95% CI = 1.00-1.05) and persistence (adjusted OR = 1.02, 95% CI = 1.01-1.04) after adjustment. Both GL and NNR were associated with greater BV prevalence and GL was associated with an increase in BV persistence and acquisition. These results suggest that diet composition may contribute to vaginal flora imbalances and be important for elucidating the etiology of BV.